Complementarity in both plant and mycorrhizal fungal communities are not necessarily increased by diversity in the other

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Abstract: 1.Higher species diversity can improve community performance within a species guild when different species complement each other in their use of the available niche, such as through resource partitioning. However, species in one guild of organisms may act as resources for another such that the diversity in one guild alters the realized niche for species in another. Yet, it remains largely untested as to whether diversity in one guild of organisms influences species complementarity in another. 2.The productivity and diversity in plant and arbuscular mycorrhizal (AM) fungal communities can be positively associated with each other through their typically mutualistic exchange of resources. Here we utilized these two interacting species guilds to determine whether greater diversity in one influences species complementarity in the other. This was done by creating monocultures and a mixture of a grass, forb, and legume in a full factorial design with monocultures and a mixture of four AM fungi. 3.The presence of AM fungi reduced differences in the performance among plant species and greater diversity of fungi generally improved plant productivity over the average of the fungal monocultures. However, plant species complementarity was not greatest with a higher diversity of fungi and was only positive with a particular fungal monoculture. 4.AM fungal abundance was not affected by plant diversity, but was greatly reduced in the grass monoculture compared to the other plant communities. Variation in fungal complementarity among plant communities was low overall and was little influenced by plant diversity. 5.Synthesis. Using a model plant-mycorrhizal system our results suggest that the composition rather than the diversity of species within one guild may be more influential in determining how species function within an associated species guild. However, our model system does not represent a broad gradient of diversity in either plant or fungal communities and only assesses the initial growth phase. Nonetheless our results highlight that changes in species compositions in one species guild can affect the functioning of species diversity in another.

DOI: https://doi.org/10.1111/1365-2745.12452

Complementarity in both plant and mycorrhizal fungal communities are not necessarily increased by diversity in the other

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Summary

1. Higher species diversity can improve community performance within a species guild when different species complement each other in their use of the available niche, such as through resource partitioning. However, species in one guild of organisms may act as resources for another such that the diversity in one guild alters the realized niche for species in another. Yet, it remains largely untested as to whether diversity in one guild of organisms influences species complementarity in another.

2. The productivity and diversity in plant and arbuscular mycorrhizal (AM) fungal communities can be positively associated with each other through their typically mutualistic exchange of resources. Here we utilized these two interacting species guilds to determine whether greater diversity in one influences species complementarity in the other. This was done by creating monocultures and a mixture of a grass, forb, and legume in a full factorial design with monocultures and a mixture of four AM fungi.

3. The presence of AM fungi reduced differences in the performance among plant species and greater diversity of fungi generally improved plant productivity over the average of the fungal monocultures. However, plant species complementarity was not greatest with a higher diversity of fungi and was only positive with a particular fungal monoculture.

4. AM fungal abundance was not affected by plant diversity, but was greatly reduced in the grass monoculture compared to the other plant communities. Variation in fungal complementarity among plant communities was low overall and was little influenced by plant diversity.

5. *Synthesis*. Using a model plant-mycorrhizal system our results suggest that the composition rather than the diversity of species within one guild may be more influential in determining how species function within an associated species guild. However, our model system does not represent a broad gradient of diversity in either plant or fungal communities and only assesses the initial growth phase. Nonetheless our results highlight that changes in species compositions in one species guild can affect the functioning of species diversity in another.

Key-words: community ecology, horizontal biodiversity effects, mutualisms, plant–soil (below-ground) interactions, vertical biodiversity effects

Introduction

The role of biodiversity has been a focal point in ecology over the past few decades as the loss of species within an ecosystem can adversely affect the ability of the ecosystem to maintain its functioning (Zavaleta *et al*. 2010; Hooper *et al.* 2012; Cardinale *et al.* 2012; Wagg *et al*. 2014). A positive biodiversity-ecosystem functioning relationship has been shown in numerous studies demonstrating that greater species diversity can enhance the overall community performance, particularly in plant communities (Balvanera *et al*. 2006; Cardinale *et al*. 2006). Such effects have also been demonstrated in various groups of organisms including bacteria (Bell *et al*. 2005; Gravel *et al*. 2011), plant symbiotic fungi (van der Heijden *et al*. 1998; Wagg *et al*. 2011a), and soil decomposers (Eisenhauer, Reich & Isbell 2012).

The relationship between species diversity, or greater species richness, and ecosystem functioning, such as net primary productivity, can be attributed to dissimilarities among species in their exploitation of the biotope (Tilman *et al*. 1997; Dimitrakopoulos & Schmid 2004; Hooper *et al*. 2005; Turnbull *et al*. 2013). Greater species diversity may increase productivity when species in more diverse communities enhance the overall resource capture of the community through niche differentiation and facilitation. For instance, including species that differ in resource acquisition strategies in more species rich communities may reduce niche overlap thereby relaxing competition and improving performance of the plant community (Tilman *et al*. 1997; Turnbull *et al*. 2013). This is commonly termed as species complementarity (Loreau & Hector 2001; Hector *et al*. 2002). On the other hand, increasing species diversity may also increase the probability of including species that are particularly productive and competitive for resources in plant mixtures. This can

result in the so-called selection effect when the higher productivity in species mixtures results from the inclusion of particularly productive species that drives the productivity of the plant mixture (Loreau & Hector 2001; Hector *et al*. 2002). Both complementarity and selection effects can lead to a greater productivity in a plant species mixtures and when summed together represent the net biodiversity effect; which is the difference of the performance of species mixtures from the average performance of the respective species in monocultures.

Since biodiversity effects can be dependent upon resource acquisition abilities of individual species, factors that alter the ability of species to acquire resources may determine whether a more diverse community functions via a complementarity or selection effect. For instance, increased biotope space belowground (e.g. increasing available rooting space) or increasing resource heterogeneity can result in a more positive effect of biodiversity on ecosystem functioning that is driven by a complementarity effect (Wacker *et al*. 2008; Dimitrakopoulos & Schmid 2004; Wagg *et al*. 2011a; Jousset *et al*. 2011). Conversely, in situations where the ability of species to differentially acquire resources is low, such as in a limiting and homogenous resource environments, increasing the number of species may consequently lead to a lower complementarity effect and/or a greater selection effect (Wagg *et al*. 2011a; Becker *et al*. 2012).

Although it is well documented that manipulating the number of species within a community will alter the functioning of that community (horizontal diversity effects), it is less understood how changes in species diversity in one guild of organisms affects the functioning of diversity in another (vertical diversity effects) (Duffy *et al*. 2007). Understanding the effect of greater diversity between guilds of

102 organisms (vertical diversity) on the functioning of an ecosystem has been recognized
103 as a key issue for identifying underlying mechanisms that affect ecosystem
104 performance. This is because the presence and diversity of species within one guild of
105 organisms can act as resources and construct niches for other guilds of organisms.
106 Therefore changes in species diversity in one guild of organisms can have cascading
107 effects across multiple guilds of organisms (McCann 2000; Silvertown 2004; Duffy *et*
108 *al*. 2007; Estes *et al*. 2011; Eisenhauer 2012).

109 The functioning of diverse plant communities can be influenced by the
110 presence and diversity of belowground organisms, such as pathogens, decomposers,
111 and mutualists, that affect resource acquisition abilities in plants and alter plant-plant
112 competition (Klironomos *et al*. 2000; Silvertown 2004; Maron *et al*. 2011; Schnitzer
113 *et al*. 2011; Wagg *et al*. 2011a; Eisenhauer, Reich & Isbell 2012; Wagg *et al*. 2014).
114 Specifically, several studies have shown that a widespread group of plant symbionts,
115 the arbuscular mycorrhizal (AM) fungi, contribute to greater productivity and
116 diversity in grassland communities by providing plants greater access to soil resources
117 (Smith & Read 2008; van der Heijden *et al.* 2015). AM fungi vary in attributes by
118 which they provide a benefit to plant hosts; from their influence on plant host
119 pathogen defence to the acquisition of various soil resources (Newsham, Fitter &
120 Watkinson 1995; Jansa, Mozafar & Frossard 2005; Powell *et al.* 2009; Thonar *et al.*
121 2011). Therefore a more diverse AM fungal community may improve plant
122 productivity by providing a variety of benefits to the host plants, such as by providing
123 plants access to different soil resource pools (Koide 2000, Jansa, Mozafar & Frossard
124 2005; Thonar *et al.* 2011, Fig. 1a,b). Additionally, a greater diversity in plant-AM
125 fungal interactions may allow for differential resource capture among plant species

126 (e.g. Wagg *et al*. 2011b, Fig. 1c,d). Therefore AM fungal diversity holds the potential
127 to influence whether plant communities exhibit a complementarity or selection effect
128 by expanding the plant available resource pool and mediating resource partitioning
129 among plants belowground (Silverton 2004; Eisenhauer 2012; and see Fig. 1).
130 However, plant species also vary in the degree to which they benefit from associating
131 with various AM fungi (Klironomos 2003). Resultantly certain AM fungi may allow
132 particular plant species greater access to soil resources than others and alter
133 competitive interactions among plant species (Fitter 1977; Urcelay & Díaz 2003;
134 Scheublin, van Logtestijn & van der Heijden 2007; Collins & Foster 2009).
135 At the same time, plant species can construct niches for soil organisms and
136 thereby alter the composition of soil biota (Bever *et al*. 1996; De Deyn *et al*. 2004;
137 Bezemer *et al*. 2010; De Deyn, Quirk & Bardgett 2011; van der Putten 2012). For
138 instance, individual plant species influence the community structure of AM fungi in
139 their rhizosphere such that different AM fungi show preference for different plant
140 hosts (Eom *et al*. 2000; Bever 2002; Burrows & Pfleger 2002; Johnson *et al*. 2003;
141 Vandenkoornhuyse *et al.* 2003; Croll *et al*. 2008). Thus, greater diversity in plant
142 hosts could potentially allow for resource partitioning among AM fungi by colonizing
143 different plant hosts such that competition among AM fungi is avoided or reduced
144 (e.g. Fig. 1b and d). In addition, AM fungi are dependent on photosynthetically
145 derived carbon from host plants to maintain hyphal development and the functioning
146 of the plant-AM fungal mutualism (Smith & Read 2008). Considering this, greater
147 plant diversity can be linked with a greater capture and allocation of carbon
148 belowground (Reich *et al*. 2001; Tilman, Hill & Lehman 2006; Steinbeiss *et al*. 2008;
149 Ladygina & Hedlund 2010), greater plant diversity could expand the available

150 resource pool for AM fungi allowing for greater AM fungal productivity and
151 furthermore increased resource partitioning among AM fungi (e.g. Fig. 1). Therefore
152 it seems likely that greater plant diversity may be a key mechanism for
153 complementarity among AM fungi.

154 Greater AM fungal diversity is well known to enhance plant community
155 productivity (e.g. van der Heijden *et al*. 1998) and plant and AM fungal diversity can
156 be associated with one another in natural grassland communities (e.g. Hiiesalu *et al*.
157 2014). However, it remains unknown as to whether the positive effect of AM fungal
158 diversity on ecosystem primary productivity results from improved resource
159 partitioning and complementarity among plant species when AM fungal diversity is
160 high. Likewise, it remains untested whether greater plant diversity is a mechanism for
161 resource partitioning among AM fungi such that greater plant diversity allows for
162 greater complementarity among AM fungi. Here we address this issue by creating a
163 model system in which we manipulated both plant and AM fungal communities to
164 create different plant diversity levels (1 or 3 plant species) crossed with different AM
165 fungal diversity levels (0, 1 or 4 AM fungi) to address the hypotheses that (*i*) greater
166 AM fungal diversity would improve plant productivity (Fig. 1) and (*ii*) if greater AM
167 fungal diversity improves plant productivity by reducing niche overlap among plant
168 species, then greater AM fungal diversity should lead to greater plant
169 complementarity (Fig. 1c, d). At the same time AM fungi may benefit from greater
170 plant diversity where we hypothesize that (*iii*) greater plant diversity improves AM
171 fungal abundance (Fig. 1a,c) and (*iv*) if a greater plant diversity reduces niche overlap
172 among AM fungi, then a greater plant diversity should improve complementarity
173 among AM fungi (Fig. 1b,d).

Materials and methods

Plants

Three plant species commonly found in natural temperate grasslands that represent major plant functional groups were used for the study; a forb (*Plantago lanceolata* L.), a grass (*Lolium multiflorum* Lam.) and a legume (*Trifolium pratense* L.). Seeds of each of these three plant species were surface sterilized in 2.5% sodium hypochlorite (50% household bleach), rinsed in distilled H_2O, and germinated on 1.5% water-agar medium for three to five days. Seedlings appearing free from contamination were transplanted into pots in either monoculture or mixture resulting in three plant monocultures and one plant mixture (4 plant community treatments). All plant communities were planted with 27 individuals in predetermined evenly spaced positions. The plant species mixture consisted of 9 individuals of each of the three plant species randomly distributed within the pot. Plants not surviving the transplantation were replaced within 2 weeks of the initial planting. Plant mortality overall was very low and was independent of plant and AM fungal treatments (see Fig. S1 in the Supporting Information).

Fungi

As with the plant species, the four AM fungal species were inoculated as a monoculture of one of the four taxa and all together as a mixture into all plant community combinations. The AM fungi were: *Funneliformis mosseae*, *Rhizoglomus irregulare*, *Claroideoglomus claroideum* and *Diversispora celata*. These AM fungi were selected based on our past experience with the isolates and the availability of the molecular tools to quantify the abundance of these fungi within a mixed AM

198 fungal community (Wagg *et al.* 2011a,b; Thonar, Erb & Jansa 2012). These fungi are
199 also abundant in Swiss grassland (e.g. Oehl *et al*. 2010). Although these fungi may
200 not represent the broad spectrum in the AM fungal phylogenies, these fungi can vary
201 in functional characteristics such as foraging strategies and effects on host plant
202 performance (Jansa, Mozafar & Frossard 2005; Thonar *et al.* 2011; Wagg *et al*.
203 2011a). It should be noted that these four fungi are the same isolates used by Wagg *et*
204 *al*. (2011a,b), but *R. irregulare, F. mosseae* and *C. claroideum* are renamed due to
205 the recent reclassification of their genera (Schüßler & Walker 2010; Krüger *et al*.
206 2012; ; Sieverding *et al.* 2014).
207 These isolates, as well as a non-mycorrhizal control inoculum, were
208 cultivated on *P. lanceolata* for 6 months in a sterilized 1: 9 field soil-quartz sand
209 mixture. Each inoculum was prepared by allowing the culture pots to dry and roots
210 were cut into small fragments and homogeneously mixed with the culture pot
211 substrate. Each experimental pot received 90 g of inoculum, comprised of colonized
212 roots and substrate containing spores and hyphae and non-AM control pots received
213 90 g of control inoculum. Pots inoculated with all four fungi received 22.5 g of each
214 AM fungal inocula, such that the total inoculum amount was 90 g per pot. In order to
215 correct for differences in microbial communities between inocula treatments, 50 g of
216 each inoculum was sieved with 800 ml of dH_2O through a sieve series with the
217 smallest being < 10 µm. The inocula washes were pooled and 20 ml was added to
218 each pot. An additional microbial wash was created using a total of 1 kg of fresh field
219 soil from a natural grass clover field with 4 L of dH_2O and sieved through a series of
220 sieves down to < 10 µm. Each pot received 20 ml of this additional microbial wash in
221 order to further standardize the microbial communities within the pots and ensure the

222 presence of rhizobia. The latter was confirmed by the presence of root nodules on *T.*
223 *pratense* in all pots at the final harvest. Moreover this did not introduce unintended
224 AM fungi from the field as no AM fungal colonization was detected in the non-AM
225 control plants.
226
227 **Experimental conditions**
228 The experiment was a complete factorial design with four plant communities
229 (three species monocultures and one mixture) and six AM fungal communities (four
230 monocultures, one mixture of all four species, and one non-AM control), which
231 resulted in a total of 24 plant-AM fungal community combinations. Each
232 combination was replicated eight times giving a total of 192 microcosms. These were
233 randomly distributed throughout the glasshouse. The communities were grown in a
234 substrate consisting of 75 % field soil and 25 % sand, which was sterilized by
235 autoclaving at 121 ˚C for 90 min and allowed to settle for two weeks prior to
236 inoculation. The field soil was collected from a natural grassland that was managed
237 only by mowing twice per year for fodder. The sterilized substrate had a pH of 7.5
238 and contained 50.5 mg/kg of water-soluble inorganic N (NO_3^- and NH_4^+). Plant
239 available P_2O_5 and K_2O (CO_2–saturated water extraction) were 0.32 mg/kg and 7.5
240 mg/kg respectively. Total Ca, P, K, and Mg (ammonium acetate-EDTA extracted)
241 were 4.26×10^3 mg/kg, 17.7 mg/kg, 24.4 mg/kg, and 161 mg/kg respectively. The
242 substrate conditions in which plants were grown may be considered to be similar to
243 some calcareous grassland soils.
244 Pots were 3 L in volume and were filled with 2500 g of sterilized substrate and
245 the 90 g of inocula was mixed throughout. Additional sterilized substrate was added

on the surface to bring the total substrate to 5000 g. The addition of the top layer was added to reduce any risk of cross contamination. The plant-fungal communities were grown in a glasshouse at the Agroscope Research Station in Zürich, Switzerland, for a total of 12 weeks. Plants received natural light subsidized by 400 W high pressure sodium lights to achieve a minimum of 300 W/m^2 of light for 16 h at 25-28 °C during the day. During the eight-hour nights plants were exposed to an average temperature of 16 °C. Pots were watered with dH_2O to maintain soil moisture between 10% and 20% by weight.

Plant and fungal data collection

Microcosms were harvested every four weeks post initial seedling transplant in order to track the development of the microcosms. At four and eight weeks post initial planting plant shoots were collected 4-5 cm above the soil surface. At 12 weeks plants were harvested at the soil surface. Shoots were sorted by species and dried at 60°C to determine biomass. Since in some pots not all individual plants survived to the end of the experiment the number of individuals of each plant species per pot was also recorded (see Fig. S1). AM fungal communities colonizing roots were also sampled at each harvest. After four and eight weeks of growth plant roots were collected by extracting three soil cores (1.4 cm in diameter) to the depth of the pot that were then refilled with the same sterile substrate. At 12 weeks entire plant community root systems were harvested. Roots were rinsed clean of soil, cut into small (1-2 cm) fragments, mixed for randomization, and a subsample was lyophilized and massed for molecular detection of AM fungi. However, at four and eight weeks the detection of AM fungi was frequently below the qPCR detection limit. Therefore

AM fungi could not be reliably detected in plant roots in a comparable manner among time points (see below for qPCR methods). For these reasons we only use AM fungal abundance data collected during the final harvest when the entire root system was sampled.

DNA was extracted from lyophilized root samples using the Qiagen DNeasy 96 plant kit following manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, MD, USA). The DNA extracted was quantified using PicoGreen® (Molecular Probes, Eugene, OR) on a Cary Eclipse Fluorescence Spectrophotometer. The nuclear large ribosomal subunit of each of the four fungi was quantified in all root samples using primers and hydrolysis probes specific to each of them. The qPCR reactions were carried out using the Light Cycler 2.0 (Roche Applied Science, Rotkreuz, Switzerland) and the cycle threshold values were used to determine the number of DNA copies per mg of lyophilized roots (see Wagg *et al*. 2011a,b and Thonar, Erb & Jansa 2012 for details). Details on the primers and probes used, as well as the reagents and cycling conditions for the qPCR are described in Wagg *et al*. (2011b) and Thonar, Erb & Jansa (2012). In addition, all non-AM control treatments were checked for AM colonization by microscopy and staining cleared roots with 5% pen ink – vinegar solution as outlined by Vierheilig *et al*. (1998). One non-AM pot of *P. lanceolata* was found to be colonized with AM fungi and thus removed from the data set.

Indices of performance and the partitioning of biodiversity effects

Total AM fungal abundance was determined as the total sum of target DNA copies detected per mg of root during the final harvest, which has been demonstrated to correlate with overall AM fungal abundance in roots (Jansa *et al*. 2014). Plant

aboveground biomass was pooled across harvests and plant species and used as the net productivity of communities in all analyses. Similarly, the biomass of each plant species was pooled separately across harvests to obtain the net productivity of each plant species. Complementarity, selection, and net biodiversity effects in the plant and fungal communities were calculated using the additive partitioning approach described by Loreau & Hector (2001), which is described in detail elsewhere (Hector *et al*. 2002; Schmid *et al*. 2008; Turnbull *et al*. 2013). Briefly, the complementarity effect was calculated as $N \times \overline{\Delta RY} \times \bar{M}$ and the selection effect was calculated as $N \times cov(\Delta RY, M)$, where N is the number of species in the mixture (i.e. 3 in the plant mixture and 4 in the AM fungal mixture) and M is the yield of the species monoculture. The relative yield (RY) is the observed yield of a species in mixture (O) divided by the species monoculture yield (M) such that $RY = O/M$ (de Wit 1960). The ΔRY is then the difference in the relative yield of a species from its expected relative yield, which is 1/N; the proportion in which the species were sown. The sum of the complementarity and selection effects is the net effect, which is the difference in the observed yield of the species mixture from the average of the species monocultures. The biodiversity effects were calculated for the plant mixture using plant species biomass and for the fungal mixture using the DNA abundance of each AM fungus.

Data analyses

All statistics were calculated using R for mac OS X version 2.15.1 (R foundation for Statistical computing, 2012). To reduce heteroscedasticity the biomass of *T. pratense* plants cube-root transformed. Additionally, all measures of fungal

abundance were log + 1 transformed prior to analyses. In all ANOVA models plant mortality was added as a covariate to counteract potential plant density effects. To assess the performance of individual plant and fungal species in their respective monocultures and mixtures, the data were standardized by the initial sown / inoculation density. Specifically, plant species biomass was assessed by using species biomass per individual per microcosm and the abundance of each AM fungus was assessed as the number of DNA copies per mg of roots sampled per mg of AM fungal inoculum. This was done in order to easily decipher monoculture and mixture effects on species performance independent of the initial intraspecific density and calculated. Therefore no plant mixture versus monoculture effect should be detected in the ANOVA model if it performs similarly in both monoculture and mixture.

To assess our hypotheses (*i*) and (*ii*) with regards to plant community performance in relation AM fungal diversity, the plant community productivity and the biomass of individual plant species were assessed by ANOVA with the plant community and AM fungal community combinations as well as their interaction as sources of variation. Contrast terms were added to the ANOVA models to test first for the effect of the presence of AM fungi versus the non-AM control plants. To explicitly test the hypothesis that a greater AM fungal diversity supports greater plant productivity (*i*), a contrast term was added to the model to compare plant productivity in the AM fungal mixture versus the AM fungal monocultures. To test the hypothesis that greater AM fungal diversity improves complementarity among plants (*ii*), we assessed the plant biodiversity effects (complementarity, selection and net effects) separately by ANOVA for variation among AM fungal communities. The contrast terms testing for the effect of the presence of AM fungi and the mixture versus

343 monocultures of AM fungi were added to the model as was done for assessing net
344 productivity. The contrast of the AM fungal mixture against the AM fungal
345 monocultures explicitly tests our hypothesis that greater AM fungal diversity alters
346 the biodiversity effects within the mixed plant species community (*ii*).
347 Total AM fungal abundance and the abundance of individual AM fungi were
348 assessed by ANOVA using plant and fungal community combinations as sources of
349 variation. Contrast terms were added to the ANOVA model to assess whether AM
350 fungal abundance in the plant mixture was greater than the plant monocultures
351 thereby explicitly testing our hypothesis that greater plant diversity supports greater
352 abundance of AM fungi (*iii*). Furthermore, AM biodiversity effects
353 (complementarity, selection and net effects) were similarly assessed with the plant
354 mixture versus monoculture contrast term added to the model to test hypothesis as to
355 whether greater plant richness improved complementarity in the abundance of AM
356 fungi (*iv*).
357

358 ## Results

359 **Effect of AM fungal communities on plants**

360 Plant communities varied strongly in net productivity among both plant and AM
361 fungal community combinations (Table 1, Fig. 2a). The overall presence of AM fungi
362 increased the productivity of the plant mixture and of the *P. lanceolata* and *T.*
363 *pratense* monocultures, while the productivity of the *L. multiflorum* monoculture was
364 generally not affected by the presence of AM fungi (Table 1, Fig. 2a). Plant
365 communities also had greater net productivity with the mixture of all four AM fungi
366 than the average of the fungal monocultures, as indicated by the contrast between the

AM fungal mixture and the average of the AM fungal monocultures (Table 1, Fig. 1a: AM fungal community M versus the average of F, R, C and D fungal treatments). The productivity of the *P. lanceolata* and *T. pratense* monocultures, as well as the plant mixture, all generally benefited from the inoculation of the individual AM fungal monocultures (Fig. 1a). However, *F. mosseae* had the lowest beneficial effect on the *P. lanceolata* and *T. pratense* monocultures and had no beneficial effect on the plant mixture (Fig. 1a). Additionally, the productivity of the *L. multiflorum* monoculture varied little among the AM fungal monocultures. This differential response in productivity of *L. multiflorum* from the other two plant monocultures to the various AM fungal monocultures resulted in a significant plant community by AM fungal treatment interaction term (Table 1). Overall, the lower productivity of plant communities with the *F. mosseae* monoculture contributed to the lower average effect of AM fungal monocultures on plant productivity relative to the AM fungal mixture.

All plant species varied in their individual performance depending on the AM fungal community and whether they were grown in monoculture or in mixture (Table 1, Fig. S1 in the Supporting Information). Overall, the grass *L. multiflorum* performed better in the plant mixture than in the plant monoculture (Table 1, Fig. 1b). Of the AM fungal monocultures, *L. multiflorum* performed best in the plant mixture with the *D. celata* monoculture resulting in significant AM by plant interaction (Table 1, Fig. 1a,b). *P. lanceolata* performed better in the plant mixture when inoculated with *C. claroideum* and worse when inoculated with *D. celata* resulting in the AM fungal by plant community interaction (Table 1, Fig. 1b). *T. pratense* performed better when grown as a plant monoculture in all AM fungal communities, although the difference between monoculture and mixture performance of *T. pratense* was not strongly

391 significant in the non-AM control treatment (Fig. 1b).

392

393 **AM fungal diversity influence on plant complementarity and selection effects**

394 As a result of the strong performance of *L. multiflorum* in both plant
395 monocultures and mixtures and the poor performance of the other two plant species
396 when AM fungi were absent, the plant species mixture out performed the average of
397 the plant monocultures indicated by a positive net biodiversity effect (Table 2, Fig. 3:
398 AM fungal community N). Since in the absence of AM fungi *L. multiflorum* was the
399 most productive species in both monoculture and mixture relative to *P. lanceolata*
400 and *T. pratense* (Fig. 2a), the positive net effect was largely driven by the selection
401 biodiversity effect, which contributed 72% of the net effect (Fig. 3). Overall, the net
402 and selection biodiversity effects in the plant species mixture were greatest in the
403 non-AM plant community than in the presence of AM fungi (Table 2, Fig. 3).

404 In the presence of AM fungi, the selection effect in the plant community did not
405 differ between the AM fungal mixture and the average of the AM fungal
406 monocultures (Table 2). However, the selection effect in the plant mixture was
407 dependent upon the identity of the AM fungal monocultures (Table 2), which was
408 largely due to *F. mosseae* resulting in a positive selection effect while *R. irregulare*
409 and *D. celata* resulted in a negative selection effect in the plant mixture (Fig. 3).

410 Overall, the plant net biodiversity effect did not vary greatly among the AM
411 fungal treatments (Table 2) and did not differ strongly from 0 in all treatments where
412 AM fungi were present (Fig. 3). Although the complementarity effect in the plant
413 community was generally positive it varied little among AM fungal community
414 treatments (Table 2) and generally did not differ from 0, with the exception of the *C.*

claroideum AM fungal monoculture (Fig. 3). With the *C. claroideum* AM fungal monoculture the plant complementarity effect was significantly positive. With this AM fungal monoculture the plant complementarity effect was 93 % of the net biodiversity effect in the plant mixture (Fig. 3 – AM fungal community C).

Effect of plant communities on AM fungi

We found the total AM fungal abundance was not greater in the plant mixture relative to the average of the plant monocultures (Table 3, Fig. 4a). The total abundance of AM fungi was generally more dependent on the plant species comprising the plant community (Table 3, Fig. 4a). This resulted from the low detection of AM fungi within the *L. multiflorum* monoculture while the *P. lanceolata* and *T. pratense* monocultures supported the highest levels of AM fungal abundance (Fig. 3). However, a significant plant community by AM fungal community interaction was detected (Table 3; Fig. 4a).

As with the total AM fungal abundance the abundance of each of the AM fungi was lowest in the *L. multiflorum* monoculture and generally greatest with the *P. lanceolata* and *T. pratense* monocultures (Table 3, also see Fig. S2 in Supporting Information). The abundance of individual AM fungi, however, did not differ between the plant mixture and the average of the plant monocultures, with the exception of *R. irregulare* where its abundance was lower in the plant species mixture than the average of the plant monocultures (Table 3, Fig. S2). There was little difference in general in the performance of individual AM fungi in monoculture and mixture (Table 3, Fig. 4b, Fig. S2). However, *F. mosseae* performed marginally

438 better in the AM fungal mixtures and *C. claroideum* performed worse in the AM
439 fungal mixtures overall (Table 3, Fig. 4b).
440
441 **Plant diversity influence on AM fungal complementarity and selection effects**
442 The plant communities had little impact on the selection, complementarity and
443 net biodiversity effects when assessed using the AM fungal abundances. Neither did
444 the biodiversity effects differ significantly from 0 (Table 4, Fig. 5). The plant species
445 mixture also showed no difference from the average of the plant monocultures in its
446 impact on the AM fungal biodiversity effects with regards to AM fungal abundance
447 (Table 4).
448
449 ## Discussion
450 It is often observed that greater AM fungal diversity can enhance the
451 productivity of plant communities (van der Heijden *et al*. 1998; Wagg *et al*. 2011a).
452 In conjunction, it has been observed that greater plant diversity can be associated
453 with greater AM fungal abundance and richness (De Deyn, Quirk & Bardgett 2011;
454 Hiiesalu *et al*. 2014). Yet, whether greater species diversity in either the plant or
455 fungal community allows for greater functional complementarity among species in
456 the other is not understood. Here we hypothesized that (*i*) greater AM fungal
457 diversity would improve plant productivity paralleling past studies and (*ii*) if greater
458 AM fungal diversity improves plant productivity by reducing niche overlap among
459 plant species, then greater AM fungal diversity should lead to greater plant
460 complementarity. As in past studies we did observe that greater AM fungal diversity
461 generally resulted in a greater plant productivity compared to the average of the AM

462 fungal monocultures in support of our hypothesis (*i*). Additionally, we found the
463 overall presence of AM fungi reduced the selection effect in the plant community
464 indicating that the productivity of the plant community was less dependent on a
465 particularly productive plant species when AM fungi are present. In contrast to our
466 hypothesis (*ii*) greater AM fungal diversity did not improve the complementarity
467 effect in the plant species mixture more than the AM fungi in monoculture average.
468 Additionally, the complementarity effect in the plant community varied little from 0
469 (no effect). However, plant complementarity was only significantly positive in the
470 presence of a single AM fungus, *C. claroideum*, indicating the identity of the plant-
471 AM fungal association may be a key determinant in plant community
472 complementarity effects.

473 On the fungal side, greater plant diversity did not improve the overall
474 abundance of AM fungi detected within plant roots, in contrast to our hypothesis (*iii*).
475 Neither were the net and complementarity biodiversity effects of the AM fungi
476 greatest in the plant species mixture. This provides little empirical evidence to
477 support our hypothesis (*iv*) that greater plant diversity reduces niche overlap among
478 AM fungi. Instead we found no evidence for AM fungal biodiversity effects based on
479 AM fungal abundance overall.

480 Our results confirm that greater AM fungal diversity can lead to greater plant
481 community performance. However, the greater plant community performance does
482 not necessarily indicate that greater AM fungal diversity reduces niche overlap
483 among plants to improve plant species complementarity. Additionally, there was little
484 evidence to suggest that greater plant diversity reduced niche overlap among AM
485 fungi. It is important to consider, however, that our experiment occurred within a

relatively short time frame (12 weeks) and biodiversity effects are known to change and develop over time (e.g. Fargione *et al*. 2007; Marquard *et al*. 2009; Reich *et al*. 2012). It is likely in our study that the effects of plant and AM fungal diversity on each other may also change through time as the plants and fungi utilize more of the available biotope and their interactions strengthen. Therefore the biodiversity effects in our study are perhaps underestimated due to the short experimental time frame. Generally, our findings highlight that although changes in species diversity in one group of organisms may impact the functioning of species diversity in an associated group of organisms, it would seem the identity of the species comprising both communities is perhaps a key determinant of vertical diversity effects (Duffy *et al*. 2007; Eisenhauer 2012; Eisenhauer *et al*. 2012).

AM fungal mediated plant community performance

We did not find greater AM fungal diversity improved plant complementarity. Instead, the complementarity effect in the plant community was only positive and contributed the most to the net biodiversity effect (93 %) when the AM fungus *C. claroideum* was present as a monoculture. Therefore an AM fungal community identity effect rather than an AM fungal diversity effect was relatively more influential on plant complementarity. In general, the effect of the AM fungal monocultures and mixture on plant performance in plant species mixtures parallels our previous findings (Wagg *et al*. 2011b) where the relative yield total of plant mixtures (RYT, which is analogous to the complementarity effect (Loreau & Hector 2001)) was greater with the monoculture of a particular AM fungus rather than a mixture of AM fungi. Similar presence and compositional effects of organisms on the

510 functioning of plant diversity have recently been observed by Eisenhauer, Reich &
511 Isbell (2012) who found the plant complementarity effect was driven more by the
512 absence and presence of groups of soil decomposers and not necessarily by their
513 diversity. Together our results provide support for the argument that community
514 composition, and not necessarily the diversity, of soil organisms determines the
515 functioning of plant communities.

516 The large net biodiversity effect in the absence of AM fungi was largely driven
517 by a positive selection effect, which is determined by species performing strongly in
518 both monocultures and mixtures (Loreau & Hector 2001). In our study, the selection
519 effect was caused by the strong productivity of *L. multiflorum* in the absence of AM
520 fungi, since both *P. lanceolata* and *T. pratense* are typically known to depend on AM
521 fungal associations for biomass production (van der Heijden *et al*. 1998; Klironomos
522 2003; Wagg *et al*. 2011a,b). The increased growth in *P. lanceolata* and *T. pratense*
523 and little variation in the performance of *L. multiflorum* in the presence of AM fungi
524 reduced the differences among the plant monoculture performances. This in turn
525 reduced the selection effect. Therefore the AM fungal communities likely played a
526 stronger role in expanding the available resource niche of *P. lanceolata* and *T.*
527 *pratense* rather than reducing niche overlap among the plants per se. Moreover,
528 competitive interactions were not strongly evident in our study as implied by the
529 species relative yields. However, in the AM fungal community treatments where *L.*
530 *multiflorum* exhibited a somewhat lower performance in the plant mixture, *P.*
531 *lanceolata* preformed relatively better suggesting a plant-plant competitive shift
532 mediated by the AM fungal communities. Such altered plant-plant competitive
533 interactions as a result of AM fungal associations parallel many past studies that have

534 also found the presence and diversity of AM fungi to mitigate competitive
535 differences among plants (Fitter 1977; van der Heijden, Wiemken & Sanders 2003;
536 Urcelay & Díaz 2003; Scheublin, van Logtestijn & van der Heijden 2007; Collins &
537 Foster 2009; Wagg *et al*. 2011b). This may suggest that the AM fungal communities
538 play some role in resource partitioning among plant species.
539
540 **Plant mediated AM fungal community performance**
541 In line with previous studies demonstrating that plant species differ in their
542 influence on AM fungi (Bever 2002; Johnson *et al*. 2003; Koch, Antunes, &
543 Klironomos 2012; De Deyn, Quirk & Bardgett 2011) we found the composition of
544 the various plant communities had a strong influence on the abundance of AM fungi
545 within the roots of the plants. Specifically, AM fungal abundance was consistently
546 lower in the *L. multiflorum* monoculture than in all other plant communities. Our
547 results compare with some previous findings that although AM fungal abundance
548 may be associated with greater plant diversity, AM fungal abundance is likely driven
549 more by the presence of particular plants species (Koch, Antunes, & Klironomos
550 2012; De Deyn, Quirk & Bardgett 2011). Such findings would indicate that greater
551 plant host diversity does not necessarily offer the AM fungi a greater opportunity to
552 increase AM fungal abundance and reduce niche overlap. Instead it suggests that the
553 resource pool available to AM fungi is largely influenced by particular plant species
554 rather than host diversity.
555 It is possible, however, that niche overlap among AM fungi is reduced by
556 colonizing different plant hosts, since plant host preference by AM fungi has been
557 observed in a number of studies (Eom *et al*. 2000; Bever 2002; Burrows & Pfleger

2002; Johnson *et al*. 2003; Vandenkoornhuyse *et al.* 2003; Croll *et al*. 2008). Moreover, spatial segregation among competing AM fungi has been observed to improve the beneficial effect of AM fungi on host plants (Bennett & Bever 2009; Bever *et al*. 2009). In our study, however, since the complementarity effect in the AM fungal mixture was not improved by greater plant host diversity we find no evidence to support the concept that spatial segregation through differential host preference among fungi occurred. Moreover, more diverse AM fungal communities are more often thought to influence plant communities through their variation in function; from improving nutrient acquisition to pathogen protection (Koide 2000; Newsham, Fitter & Watkinson 1995; Powell *et al*. 2009). Additionally, AM fungi have been shown to differ in their hyphal growth strategies and spatial foraging for soil phosphorus (Hart & Reader 2002; Jansa, Mozafar & Frossard 2005; Thonar *et al*. 2011). Therefore, spatial niche segregation within the soil matrix, rather than host plant associations, may be relatively more important for facilitating reduced niche overlap among AM fungi.

Conclusions

Overall, the presence and diversity of AM fungi improved the productivity in the plant communities. However, it did not necessarily increase the complementarity in the plant species mixture. This indicates that greater AM fungal diversity may not improve the net plant community productivity by reducing niche overlap among plant species. Instead the presence, and to a lesser extent the identity of the AM fungi, influenced the biodiversity effects in the plant community by expanding the resource niche available to the forb and legume to reduce the plant selection effect. On the

582 fungal side, the abundance of AM fungi was heavily influenced by a particular plant
583 species and not by plant diversity indicating that a greater plant host diversity does
584 not necessarily and always reduce AM fungal niche overlap.
585 Here the lack of strong effects of plant or AM fungal diversity on the
586 community functioning of the other guild does not support our hypotheses (*ii)* and
587 (*iv*). However, the weak differences in the abundance of AM fungi between the AM
588 fungal mixture and monocultures, coupled with the generally weak plant biodiversity
589 effects in the plant community in the presence of AM fungi could indicate that the
590 plant and AM fungal communities were still in the early stages of establishment. This
591 may be due to the larger belowground rooting volume and shorter time frame than
592 our previous studies (e.g. Jansa, Smith & Smith 2008; Wagg *et al*. 2011a) such that
593 the short timeframe used here did not allow for the fungi and plants to fully
594 proliferate, establish and interact within our system. Furthermore, our model system
595 does not represent a broad gradient of diversity in either plant or fungal communities
596 that may have also contributed to the generally low biodiversity effects. Therefore it
597 will be important to assess a broader depth of species diversity as well as the
598 temporal development of the plant-AM fungal diversity interaction in the future.
599 Generally, our results reinforce that the functioning of a more diverse
600 community does not only depend upon its interactions with neighbouring species of
601 the same guild, but also on the composition of associating guilds of organisms (Duffy
602 *et al*. 2007, Eisenhauer 2012, Eisenhauer, Reich & Isbell 2012). Therefore changes in
603 species compositions in either or both plant and soil communities may be key
604 components to understanding the effect of species losses on the overall functioning of
605 the ecosystem (Hodge & Fitter 2013, van der Putten 2012).

606

607 **Acknowledgements**

608 We thank Caroline Scherrer for the maintenance and preparation of the AM
609 fungal inocula and Beat Boller for providing seeds of the plant species. CW and
610 MvdH were supported by Agroscope. CW was further supported by the Jena
611 Experiment (DFG grant FOR456/1451) and by the EU project SOLIBAM. JJ was
612 supported by the Fellowship J.E. Purkyně and by the long-term development program
613 RVO61388971. We thank Dr. Michael O'Brien for comments on earlier versions of
614 the manuscript. C.W. We thank the three anonymous reviewers for providing many
615 insights into improving the manuscript.

616

617 **Data accessibility**

618 Data are available at: DOI: doi:10.5061/dryad.g464v

619

620 **References**

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1 Biomass and mean survival of individual plant species.

Figure S2 Abundances of individual AM fungi.

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TABLES

Table 1. ANOVA results for net plant community productivity (NPP) and the average biomass per plant harvested of each plant species. Mean squares (MS) and the associated degrees of freedom are shown in parentheses for effect of the different plant communities (Plant), AM fungal communities (AM), and their interaction (AM × Plant). Indented terms indicate contrast terms where NM represents the differences between the non-AM control and the treatments with AM fungi. The Div term indicates the contrast between the mixture of AM fungi and the average of the four fungal monocultures (diversity effect of AM fungal richness on plant productivity)

	NPP		*L. multiflorum*		*P. lanceolata*		*T. pratense*	
	MS	F	MS $\times 10^2$	F	MS $\times 10^2$	F	MS $\times 10^2$	F
Plant	20.4 (3)	19.5 ***	70.0 (1)	104. ***	4.01 (1)	14.8 ***	20.5 (1)	131. ***
NM	430. (1)	411. ***	0.56 (1)	0.84	56.5 (1)	208. ***	129. (1)	824. ***
Div	20.6 (1)	19.7 ***	0.22 (1)	0.32	3.17 (1)	11.7 **	2.57 (1)	16.4 ***
AM	63.7 (3)	61.0 ***	4.67 (3)	6.95 ***	11.0 (3)	40.4 ***	6.64 (3)	42.2 ***
NM × Plant	60.1 (3)	57.5 ***	2.27 (1)	3.38 †	0.02 (1)	0.06	1.17 (1)	7.44 **
Div × Plant	2.14 (3)	2.05	1.37 (1)	2.04	0.02 (1)	0.05	0.04 (1)	0.27
AM × Plant	5.86 (9)	5.61 ***	2.38 (3)	3.54 *	2.18 (3)	8.00 ***	0.24 (3)	1.51
Residual	1.04 (166)		0.67 (83)		0.27 (82)		0.16 (83)	

† P<0.1, * P<0.05, ** P<0.01, *** P<0.001

Table 2. ANOVA results for the complementarity effect (CE) selection effect (SE) and the net effect (NE) in the plant mixture. Mean squares (MS) and the associated degrees of freedom are shown in parentheses. Indented terms indicate contrast terms where NM is the contrast between the non-AM control and the treatments with AM fungi and Div is the contrast between the AM fungal mixture and the average of the AM fungal monocultures

		SE		CE		NE	
	df	MS	F	MS	F	MS	F
NM	1	13.9	203. ***	0.8	0.08	16.1	16.0 ***
Div	1	0.09	1.24	1.04	1.06	1.71	1.70
AM	3	1.03	15.0 ***	0.62	0.64	0.59	0.58
Residual	4	0.07		0.97		1.01	

MS = Mean Squares, df = degrees of freedom, F = F statistic,† P<0.1, * P<0.05, ** P<0.01, *** P<0.001

867 **Table 3.** ANOVA results for total log+1 transformed AM fungal abundance and the abundance of each fungal species. Mean squares (MS) and
868 the associated degrees of freedom are shown in parentheses for effect of the different plant communities (Plant), AM fungal communities (AM),
869 and their interaction (AM × Plant). Indented terms (Div and Div × AMF) indicate contrast terms for assessing the difference between the plant
870 mixture and average of the plant monocultures (diversity effect of plant species richness on total fungal community abundance)

871

	Total		*F. mosseae*		*R. irregulare*		*C. claroideum*		*D. celata*	
	MS	F	MS $\times 10^{1}$	F	MS	F	MS $\times 10^{1}$	F	MS $\times 10^{1}$	F
Div	0.54 (1)	0.13	1.22 (1)	0.14	3.76 (1)	5.66 *	6.63 (1)	2.87 †	0.94 (1)	0.38
Plant	255. (2)	59.2 ***	75.3 (2)	8.58 ***	11.3 (2)	17.0 ***	50.1 (2)	21.7 ***	24.1 (2)	9.69 ***
AM	3.08 (4)	0.72	26.3 (1)	3.00†	0.41 (1)	0.62	10.9 (1)	4.72 *	1.50 (1)	0.60
Div × AM	4.02 (4)	0.93	0.02 (1)	< 0.00	0.14 (1)	0.21	1.09 (1)	0.47	3.95 (1)	1.58
Plant × AM	10.5 (8)	2.44 *	2.85 (2)	0.32	0.13 (2)	0.20	4.07 (2)	1.76	3.76 (2)	1.51
Residual	4.31 (139)		8.77 (55)		0.66 (55)		2.31 (55)		2.49 (55)	

872 † P<0.1, * P<0.05, ** P<0.01, *** P<0.001

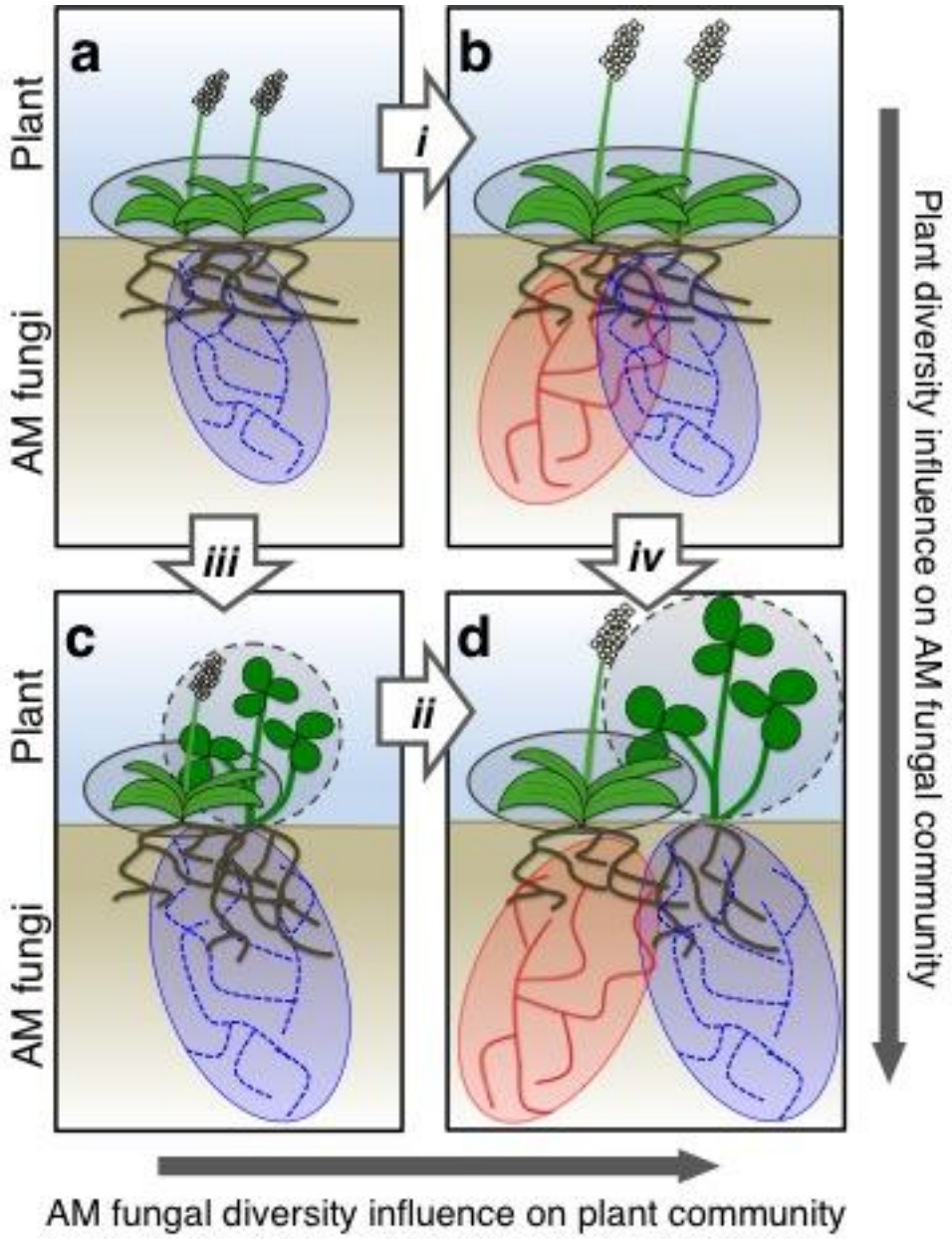
873 **Table 4.** ANOVA results for the complementarity effect (CE) selection effect (SE)
874 and the net effect (NE) in the AM fungal mixture. Mean squares (MS) and the
875 associated degrees of freedom are shown in parentheses. The indented term Div is the
876 contrast between the plant mixture and the average of the plant monocultures

		SE		CE		NE	
	df	MS	F	MS	F	MS	F
Div	1	0.04	0.002	6.85	0.79	65.3	0.74
Plant	2	43.7	2.63	4.31	0.50	14.7	1.66
Residual	27	16.6		8.68		8.86	

877 MS values are $\times 10^7$

878

879 **FIGURES**



880

881 **Figure 1.** Depiction of four hypothetical plant and AM fungal communities with
882 differing levels of plant and AM fungal diversity. Panels (a) and (b) represent a low
883 plant diversity, while (c) and (d) represent high plant diversity. Panels (a) and (c)
884 represent low AM fungal diversity, while (b) and (d) represent high AM fungal
885 diversity. The niche space occupied by each plant is indicated by the solid and dashed
886 outlined grey ellipses and the niche space occupied by each AM fungus is represented

887 by the blue and red shaded elipses to illustrate the hypotheses that (*i*) greater AM
888 fungal diversity utilizes more of the belowground niche and resultantly improves
889 plant productivity, (*ii*) greater AM fungal diversity reduces niche overlap among
890 interspecific plants by expanding the available belowground resource niche and
891 allowing for greater resource partitioning, (*iii*) greater plant diversity expands the
892 available niche for AM fungi belowground allowing for greater AM fungal
893 abundance, (*iv*) greater plant diversity reduces niche overlap among AM fungi by
894 diversifying the belowground biotope and expanding the available resource niche.

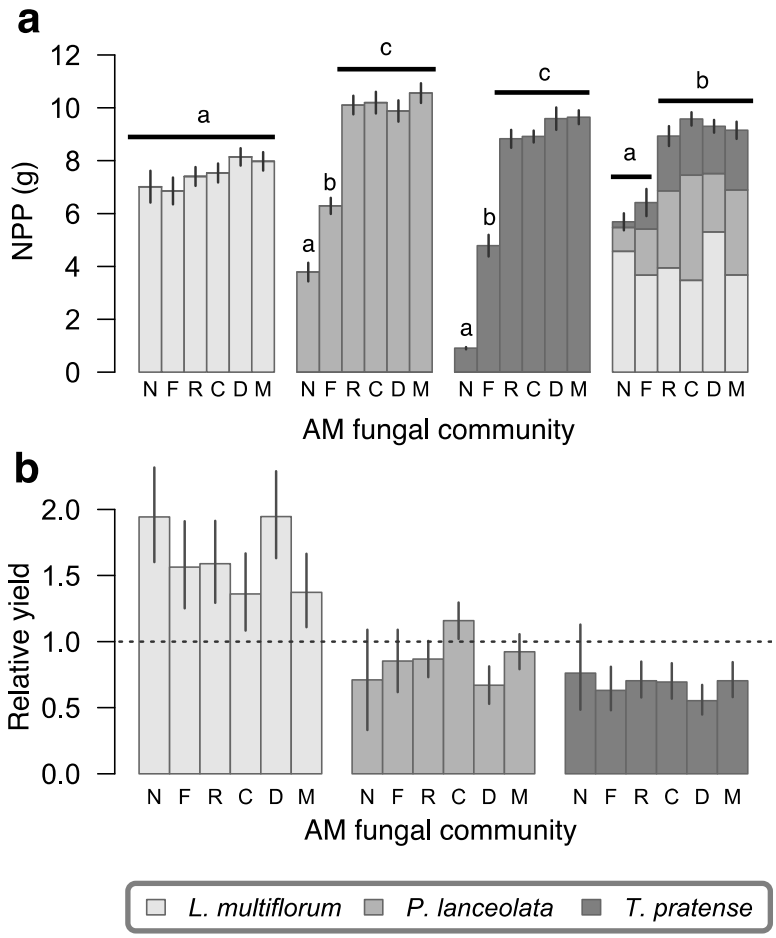
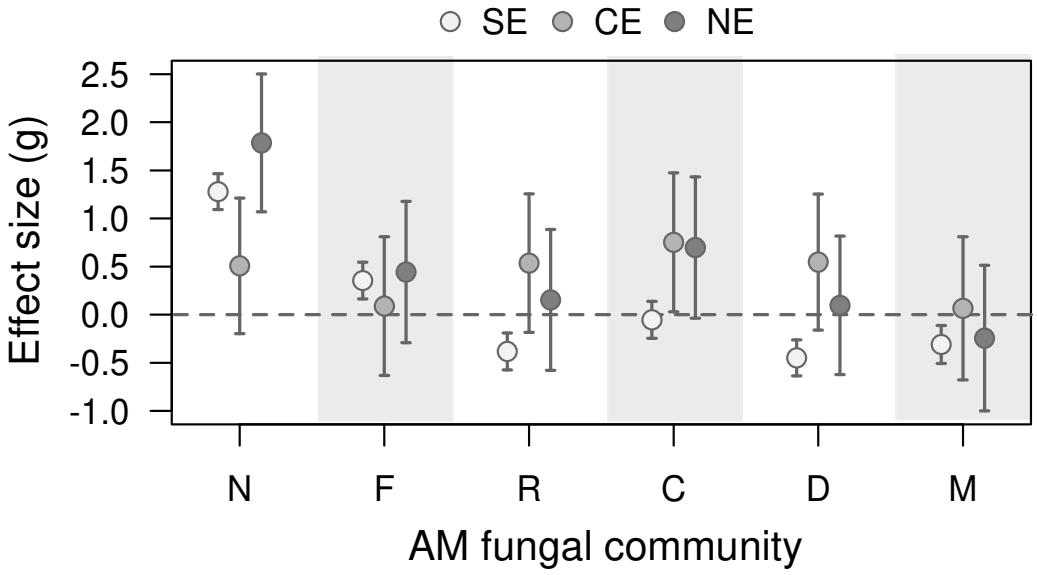


Figure 2. Mean net plant productivity (NPP) with standard errors for each plant and AM fungal community combination is shown in (a). Different lowercase letters indicate differences in net productivity among AM fungal treatments for each plant community (Tukey's HSD, $P < 0.05$). The mean performance of each plant species per sown density in mixture relative to the monoculture is expressed as a relative yield in (b) with 95 % confidence intervals for the difference from monoculture (indicated by the dotted line. Note the standardizing of yields by sown density means monoculture and mixture yields are equal when the relative yield = 1). Shading indicates different plant species, such that the stacked bars in (a) is the biomass of each plant species within the plant mixture (also see Fig. S1). The AM fungal community composition is indicated by uppercase letters on the x-axis: N = non-AM

907 control, F = *F. mosseae*, R = *R. irregulare*, C = *C. claroideum*, D = *D. celata* and M

908 = AM fungal mixture.



909

910 **Figure 3.** Plant biodiversity effects are shown for each AM fungal treatment. Points

911 indicate means and error bars indicate 95% confidence intervals for a difference from

912 0 (dashed line – no biodiversity effect) for the selection (SE = open points),

913 complementarity (CE = lightly shaded points), and the net (NE = darkly shaded

914 points) effects. The AM fungal communities are indicated on the x-axis by N = non-

915 AM control, F = *F. mosseae*, R = *R. irregulare*, C = *C. claroideum*, D = *D. celata* and

916 M = AM fungal mixture.

917

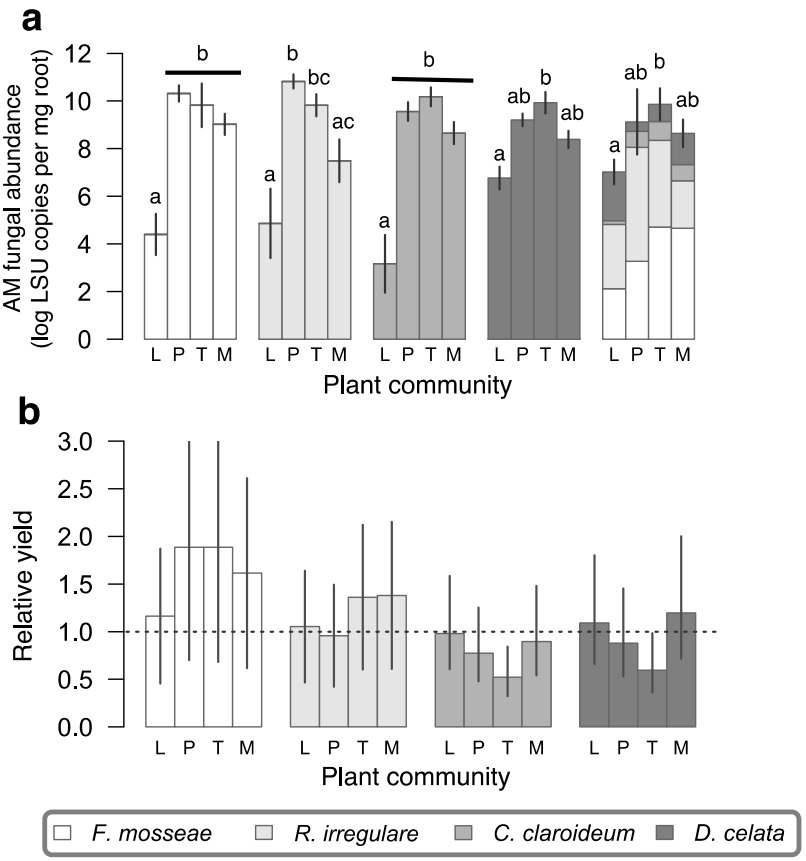


Figure 4 Mean AM fungal abundance (log transformed) with standard errors for each plant and AM fungal community combination is shown in (a). Different lowercase letters indicate differences in AM fungal abundance among plant communities within each AM fungal treatment (Tukey's HSD, $P < 0.05$). The mean performance of each AM fungi per inoculum volume in mixture relative to the monoculture is shown in (b) and is expressed as the relative yield with 95 % confidence intervals for the difference from the fungal monoculture (indicated by the dotted line. Note the standardizing of AMF abundance by inoculum volume means monoculture and mixture yields are equal when the relative yield = 1). Shading indicates the different AM fungi, such that the stacked bars in (a) indicate the proportional abundance of each AM fungus in the AM fungal mixture (also see Fig. S2). The plant community composition is indicated

by different uppercase letters on the x-axis where L = *L. multiflorum*, P = *P. lanceolata*, T = *T. pratense* and M = plant species mixture.

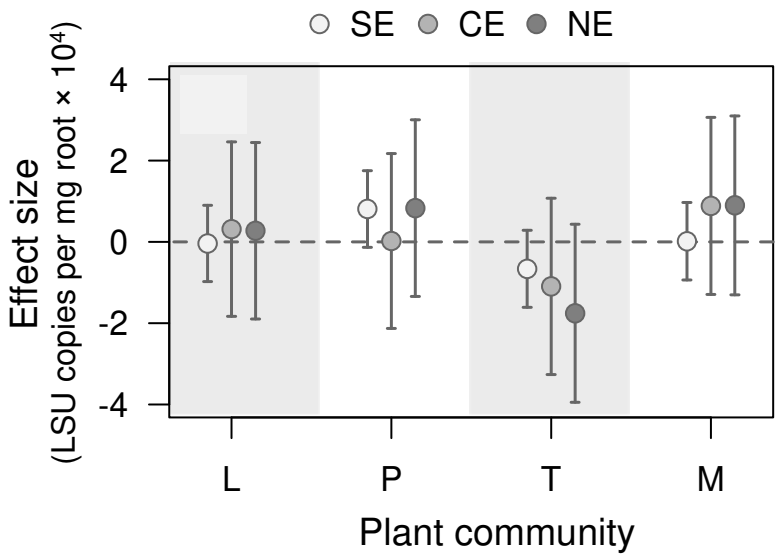


Figure 5. AM fungal biodiversity effects on the total abundance of AM fungi are shown for each plant community treatment. Points indicate means and error bars indicate 95% confidence intervals for a difference from 0 (dashed line – no effect) for the selection (SE = open points,), complementarity (CE = lightly shaded points), and the net (NE = darkly shaded points) effects. The plant communities are indicated on the x-axis by L = *L. multiflorum*, P = *P. lanceolata*, T = *T. pratense* and M = plant species mixture.